

EAST Search History

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|-------|------|------------------------------------|---|------------------|---------|------------------|
| L1 | 1 | 10/659326 | US-PGPUB; USPAT; EPO; JPO; DERWENT | OR | ON | 2007/01/05 10:05 |
| L2 | 45 | CCCCAA | US-PGPUB; USPAT; EPO; JPO; DERWENT | OR | ON | 2007/01/05 10:11 |
| L3 | 3 | linear chromosome splitting vector | US-PGPUB; USPAT; EPO; JPO; DERWENT | WITH | ON | 2007/01/05 10:10 |
| L4 | 6 | Harashima Satoshi | US-PGPUB; USPAT; EPO; JPO; DERWENT | NEAR | ON | 2007/01/05 10:10 |
| L5 | 32 | I2 and chromosome | US-PGPUB; USPAT; EPO; JPO; DERWENT | OR | ON | 2007/01/05 10:16 |
| L6 | 29 | I2 and yeast | US-PGPUB; USPAT; EPO; JPO; DERWENT | OR | ON | 2007/01/05 10:16 |

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(FILE 'HOME' ENTERED AT 10:39:36 ON 05 JAN 2007)

FILE 'MEDLINE, SCISEARCH, CAPLUS, BIOSIS' ENTERED AT 10:44:15 ON 05 JAN 2007

L1 E HARASHIMA SANTOSHI/AU
198 S E4
E SUGIYAMA M/AU
E SUGIYAMA MINETAKA/AU
L2 34 S E3
L3 24 S L1 AND L2
L4 11 DUP REM L3 (13 DUPLICATES REMOVED)
L5 33 S CCCCCA
L6 4 S L5 AND YEAST
L7 2 DUP REM L6 (2 DUPLICATES REMOVED)
L8 18 S SPLIT? CHROMOSOME (L) YEAST
L9 6 DUP REM L8 (12 DUPLICATES REMOVED)
L10 6 SORT L9 PY

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L4 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2007 ACS on STN
TI Linear chromosome splitting vector comprising target sequence, marker gene
 or centromere sequence and (C4A2)n sequence for modifying yeast
 chromosomes
SO Eur. Pat. Appl., 49 pp.
 CODEN: EPXXDW
IN Harashima, Satoshi; Sugiyama, Minetaka; Kaneko,
 Yoshinobu
AB The present invention provides a method of modifying yeast chromosomes
 using linear chromosome splitting vectors. The method of the invention
 includes preparing a first linear chromosome splitting vector comprising a
 first target sequence, a marker gene sequence, and a first (C4A2)n
 sequence; preparing a second linear chromosome splitting vector comprising a
 second target sequence, a centromere sequence of a chromosome, and a
 second (C4A2)n sequence; and introducing the chromosome splitting vectors
 into a cell, wherein n is independently an integer of 1 to 30, preferably
 4-15, more preferably 6-10. The invention relates to PCR and primers for
 construction of chromosome splitting vectors. Yeast chromosome could be
 split sequentially into five chromosomes.

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|----|--|------|----------|-----------------|----------|
| PI | EP 1422295 | A1 | 20040526 | EP 2003-256936 | 20031103 |
| | R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK | | | | |
| | JP 2004166654 | A | 20040617 | JP 2002-339259 | 20021122 |
| | US 2004224415 | A1 | 20041111 | US 2003-659326 | 20030911 |

L4 ANSWER 6 OF 11 MEDLINE on STN DUPLICATE 5
TI A versatile and general splitting technology for generating targeted YAC
 subclones.
SO Applied microbiology and biotechnology, (2005 Nov) Vol. 69, No. 1, pp.
 65-70. Electronic Publication: 2005-10-20.
 Journal code: 8406612. ISSN: 0175-7598.
AU Kim Yeonhee; Sugiyama Minetaka; Yamagishi Kazuo; Kaneko
 Yoshinobu; Fukui Kiichi; Kobayashi Akio; Harashima Satoshi
AB Yeast artificial chromosomes (YAC) splitting technology was developed as a
 means to subclone any desired region of eukaryotic chromosomes from one
 YAC into new YACs. In the present study, the conventional YAC splitting
 technology was improved by incorporating PCR-mediated chromosome splitting
 technique and by adding autonomously replicating sequence (ARS) to the
 system. To demonstrate the performance of the improved method, a 60-kb
 region from within a 590-kb YAC (clone CIC9e2 from Arabidopsis thaliana

chromosome 5) that could not be subcloned using the original method was split to convert into a replicating YAC. Two template plasmids, pSK-KCA and pSKCLY, were used to generate two splitting fragments by PCR. Two splitting fragments consisted of telomeric (C(4)A(2))(6) repeats, 400-bp target region, CEN4, H4ARS and Km(r) (selective marker for plant transformants), or CgLEU2. These splitting fragments were introduced into *Saccharomyces cerevisiae* harboring the 100-kb split YAC generated by splitting of the 590-kb YAC and containing the 60-kb region. Among 12 Leu(+) transformants, four exhibited the expected karyotype in which two newly split 40- and 60-kb chromosomes were generated. These results demonstrate that the improved method can convert a targeted region of a eukaryotic chromosome within a YAC into a replicating YAC.

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